Epidermal Damage Induced by Irritants in Man: A Light and Electron Microscopic Study

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Irritant contact dermatitis may be induced by many chemicals and has a far greater incidence than allergic contact dermatitis. Despite this, it receives relatively little attention and its pathogenesis remains poorly understood. To gain a greater understanding of the interaction of irritants with the skin, we investigated the histopathological changes resulting from the topical application of a series of structurally unrelated irritants. Human volunteers were patch-tested with appropriate concentrations of nonanoic acid, sodium lauryl sulphate, dithranol, benzalkonium chloride, croton oil, and propylene glycol, which produced generally mild to moderate responses. Biopsy specimens were taken after 48 h and examined by light and electron microscopy. Spongiosis and the infiltration of predominantly mononuclear cells were observed in the epidermis of the majority of biopsy specimens, and were particularly pronounced and extensive in croton oil reactions. In addition, several irritants induced distinct and characteristic patterns of keratinocyte damage. Nonanoic acid and sodium lauryl sulphate caused morphologic changes indicative of disturbances in keratinocyte metabolism and differentiation, giving rise to dyskeratosis and parakeratosis respectively, while dithranol induced marked swelling of keratinocytes in the upper epidermis. The results suggest that there is a diversity and specificity in the histopathology of irritant contact dermatitis, reflecting the different ways in which chemicals may interact with components of the skin. *J Invest Dermatol 93:695–699, 1989*

Many chemicals are known to induce irritant contact dermatitis in humans, and a number are capable of doing so after single exposure, if applied long enough at sufficiently high concentration. Such primary irritants show great diversity with respect to chemical structure, molecular weight, polarity and binding capacity, and as a consequence may interact with and cause damage to the cellular components of the skin in a variety of different ways. There may be denaturation of epidermal keratins, the removal of surface lipids and water holding substances, damage to cell membranes, and/or direct cytotoxic effects [1–7].

The histopathological changes that accompany this spectrum of damage have been widely studied [8–16] and there is some evidence to suggest that they vary in accordance with the mode of action of the irritant.* In an electron microscopic study, Nagao et al [17] demonstrated that topical application of sodium hydroxide and hydrochloric acid resulted in different ultrastructural changes to the epidermis. Similarly, the lipid solvents acetone and kerosene were found to induce different morphologic features of cellular damage [18]. However, systematic investigation of the pathological effects of a range of different irritants on human skin has rarely been performed. Therefore we decided to study the responses to six chemically unrelated irritants after a 48-h exposure, using both light and electron microscopy. Representative irritant chemicals were chosen from among the principal groups of primary irritants, including detergents, solvents, oils, and medicaments and these were applied at previously determined concentrations so as to induce only mild to moderate reactions [19]. The patterns of damage that they induced in the epidermis are described herein.

METHODS

A panel of ten healthy, nonatopic male volunteers with no history of skin disease participated in the study. Their ages ranged from 18–61 yr, with a mean age of 35 yr. Approval for the study was obtained from the Wycombe Ethical Research Committee and all patients gave informed, written consent.

The following irritants were tested: 0.5% (w/v) aqueous benzalkonium chloride, 5% (w/v) aqueous sodium lauryl sulphate, 0.8% (w/w) croton oil in yellow soft paraffin, 80% (w/v) nonanoic acid in propan-1-(w/w) 100% propylene glycol, and 0.02% (w/w) dithranol in yellow soft paraffin. Controls of distilled water, yellow soft paraffin, propan-1-(w/w) and occlusion only were also included.

Each volunteer was patch-tested with all of the irritants and two of the four controls. Test substances were applied to the volar aspect of the forearm, using 8-mm Finn Chambers (Epitest Ltd, Oy, Helsinki, Finland) secured by Scanpore tape. Those in solution were placed on filter paper discs inserted into the chamber, using 15 μl/Finn Chamber. Samples in yellow soft paraffin were applied directly to the chamber surface, using 25 mg/Finn Chamber. After 48 h, the patch tests were removed and 1 h later the degree of response at each site was visually assessed by two observers. Reactions were graded as negative, mild (erythema alone), moderate (erythema with edema), or severe (erythema, edema, and vesiculation). Punch biopsy specimens (4 mm) were taken, using 2% lignocaine as local anesthetic, after which the skin samples were bisected and prepared for microscopy. One sample of normal skin from an area adjacent to the patch test sites also was taken from each volunteer.

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* Björnberg A: Skin reactions to primary irritants in patients with hand eczema. An investigation with matched controls (MD thesis). Goteborg, Oscar Isaacsons Trycker AB, 1968.

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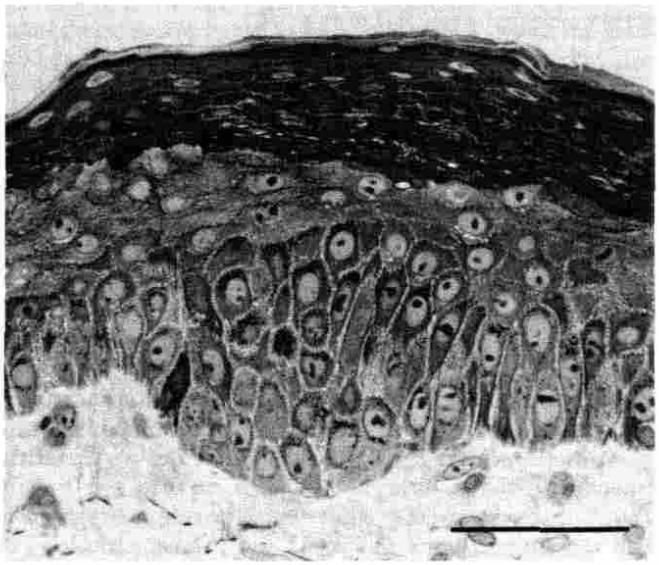

Figure 1. Parakeratosis induced by sodium lauryl sulphate. Toluidine blue-stained 1-μm semithin section (*bar* = 50 μm).

One-half of each biopsy was snap frozen in liquid nitrogen. Cryostat sections (4 μm) were cut from five different areas of each sample and stained with H & E. The remaining pieces were fixed in 3% glutaraldehyde and embedded in araldite resin as described [20]. Semi-thin sections (1 μm) were cut and stained with toluidine blue for light microscopic examination. Ultrathin sections were cut using a Reichert Ultracut, stained with lead citrate and examined under a Jeol 100CX transmission electron microscope operated at 80KV. All sections were examined in a blinded fashion.

RESULTS

All control patch tests were negative by visual assessment and, with the exception of one distilled water and two yellow soft paraffin biopsies that had occasional small areas of mild spongiosis, there were no pathological changes on histologic examination.

Clinical responses to sodium lauryl sulphate were judged clinically as mild [4] to moderate [5] with one severe reaction. When examined by light microscopy the predominant histopathologic feature in the majority of biopsies was parakeratosis, varying in thickness between samples and consisting of flattened, eosinophilic cells (Fig 1). On ultrastructural examination, the cells were found to have dense, osmiophilic cytoplasm containing numerous lipid droplets and membrane-bound vesicles (Fig 2). Many of those situated immediately beneath the stratum corneum possessed unusual nuclei with centrally located homogeneously staining chromatin. Keratohyalin granules were absent from these parakeratotic cells and from the keratinocytes in the stratum granulosum below. Keratinocytes within the stratum spinosum also contained numerous small vesicles and lipid vacuoles. Spongiosis was present in the majority of biopsies but was generally patchy and relatively mild with minimal infiltration of mononuclear cells into the epidermis. In contrast, the severe reaction caused severe necrolysis of the basal layers of the epidermis with loss of the dermoepidermal junction, accompanied by extensive infiltration of polymorphonuclear leukocytes into the epidermis and dermis.

Nonanoic acid induced mild [6] to moderate reactions [4] as judged by visual criteria. Histologically, the most notable feature in all samples was the presence of "tongues" of dense, irregularly shaped eosinophilic keratinocytes containing shrunken nuclei that extended downwards from the stratum granulosum into the stratum spinosum (Fig 3). When examined by electron microscopy the cytoplasm of these cells was found to be largely composed of dense wavy aggregates of osmiophilic keratin filaments, associated with prominent intercellular desmosomes (Fig 4). Lipid vacuoles and

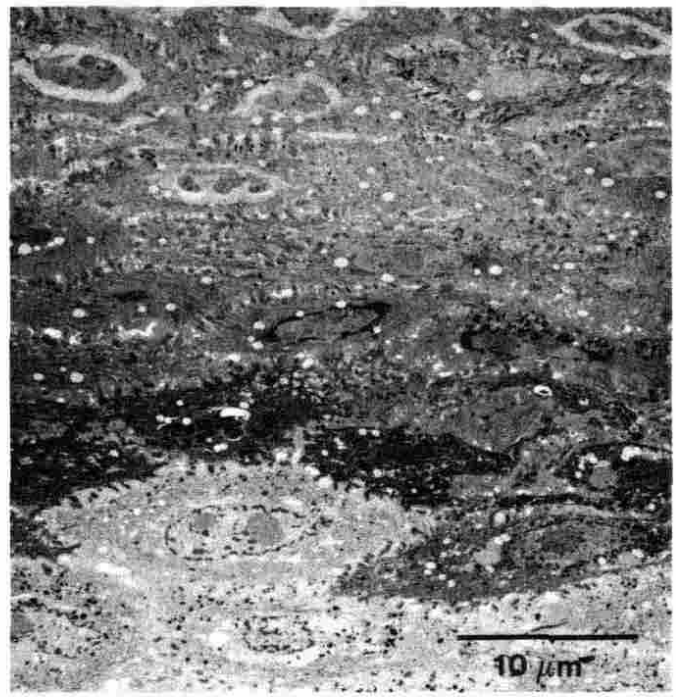

Figure 2. Low-power transmission electron micrograph of sodium lauryl sulphate reaction showing parakeratotic cells with dense osmiophilic cytoplasm containing lipid droplets and membrane bound vesicles, but devoid of keratohyalin granules. Cells immediately beneath the stratum corneum possess unusual nuclei with centrally located, homogeneously stained chromatin.

membrane-bound vesicles were present in varying numbers among the filaments. The nuclei were reduced in size and contained condensed, darkly staining marginated heterochromatin. Interspersed between the abnormal cells in the stratum granulosum were keratinocytes of more typical appearance, containing keratohyalin granules of normal structure and distribution. Cells within the stratum spinosum and basal layer were largely unaffected, except for the presence of occasional lipid droplets. Spongiosis was minimal, with few infiltrating mononuclear cells.

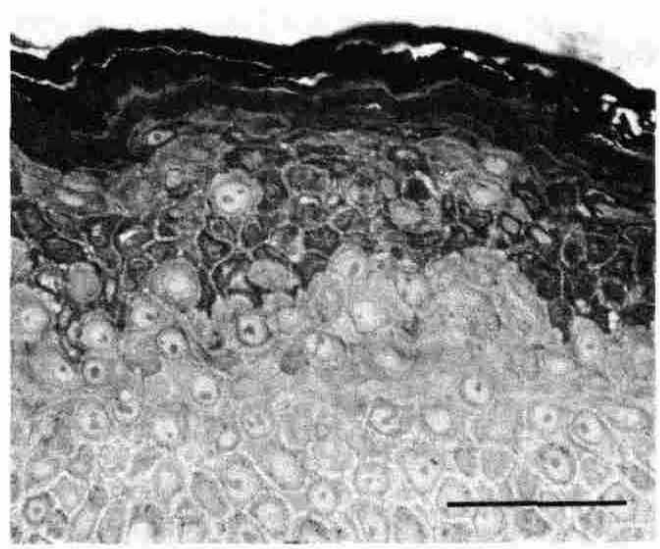

Figure 3. Nonanoic acid reaction showing "tongues" of dyskeratotic cells extending from the stratum granulosum into the stratum spinosum. Toluidine blue-stained 1-μm semithin section (*bar* = 50 μm).

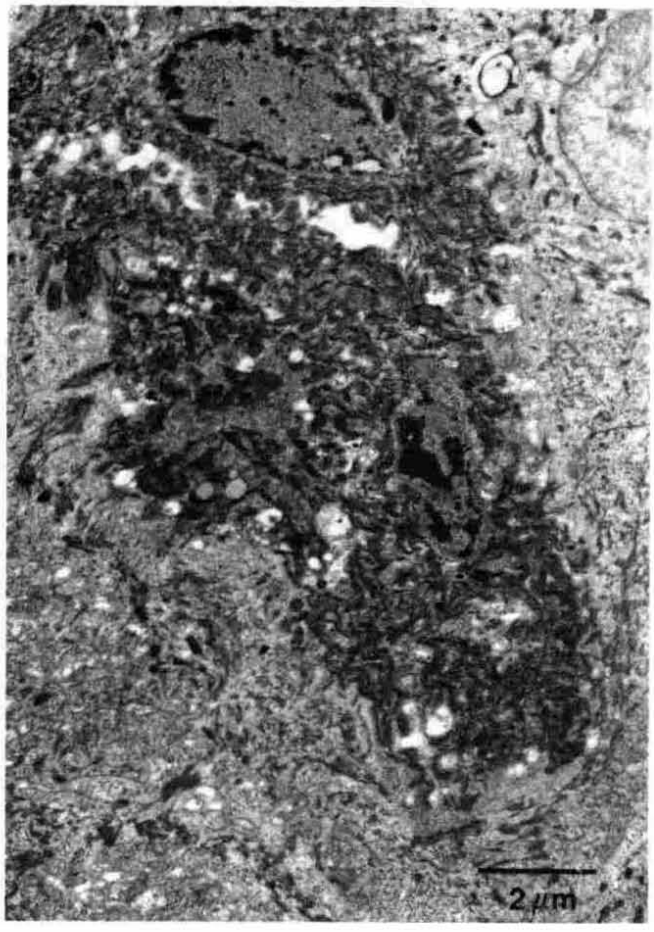

Figure 4. Ultrastructural appearance of dyskeratotic cells produced by nonanoic acid. The cytoplasm is largely composed of aggregates of osmiophilic keratin filaments, associated with prominent intercellular desmosomes.

Patch tests with propylene glycol were negative on visual assessment. By light microscopy, however, a striking "basketweave" pattern to the stratum corneum was evident in all biopsies (Fig 5). In addition, approximately half contained occasional areas of slight spongiosis, accompanied by a minimal infiltrate of mononuclear cells into the epidermis. Electron microscopy showed nonspecific changes associated with this intercellular edema.

Reactions to benzalkonium chloride were predominantly mild [6] with three negative reactions and one moderate response. Examination by light microscopy showed mild, patchy spongiosis in the majority of samples, with exocytosis of small numbers of predominantly mononuclear cells (Fig 6). Occasional foci of necrotic damage were evident in the upper stratum spinosum. Ultrastructurally, the affected keratinocytes were characterized by shrunken pyknotic nuclei, disrupted organelles, and membranes and considerable intracytoplasmic vacuolation (Fig 7). Lipid accumulation within keratinocytes was rarely observed.

The application of croton oil produced reactions that were visually assessed as being mild [4], moderate [4], and severe [2]. Histopathologically, the major features were spongiosis and exocytosis, both of which were often pronounced and extensive even in mild reactions. Mononuclear cells formed the major component of the infiltrate in the mild and moderate reactions, and were frequently observed within small intercytoplasmic vesicles in the upper epidermis. In the two severe reactions considerable spongiosis had been induced, leading to the formation of large intraepidermal bullae containing significant numbers of polymorphonuclear leukocytes, as well as mononuclear cells and flattened acantholytic keratinocytes (Fig 8). By electron microscopy, keratinocytes surrounding

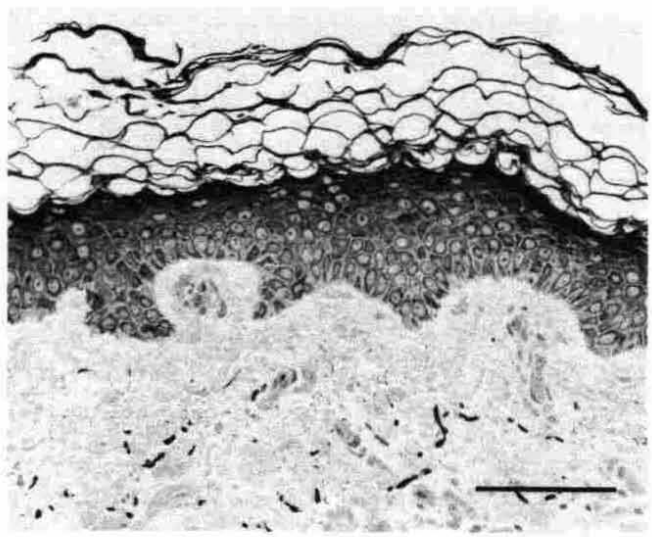

Figure 5. Hydration of corneal cells by propylene glycol, producing "basketweave" pattern to the stratum corneum. Toluidine blue-stained 1-μm semithin section (*bar* = 100 μm).

the bullae were shown to be swollen and vacuolated, with either pyknotic or enlarged nuclei. As in the benzalkonium chloride reactions, lipid vacuoles were rarely present in the cytoplasm of the epidermal cells.

The majority of dithranol reactions were graded clinically as moderate, with three being assessed as mild responses. When examined by light microscopy, all biopsy specimens showed some degree of spongiosis, particularly in the basal layers of the epidermis. This varied in severity but was most commonly mild and patchy and accompanied by a sparse infiltrate of mononuclear cells. In addition to this, markedly swollen, palely staining keratinocytes were present in the stratum granulosum and upper stratum spinosum of the majority of the biopsies from moderate reactions (Fig 9). At the ultrastructural level most of these cells were found to contain finely dispersed filaments and ribosomes, and were devoid of keratohyalin granules (Fig 10). Mitochondria, where present, had disrupted internal membranes, and were frequently clustered around enlarged

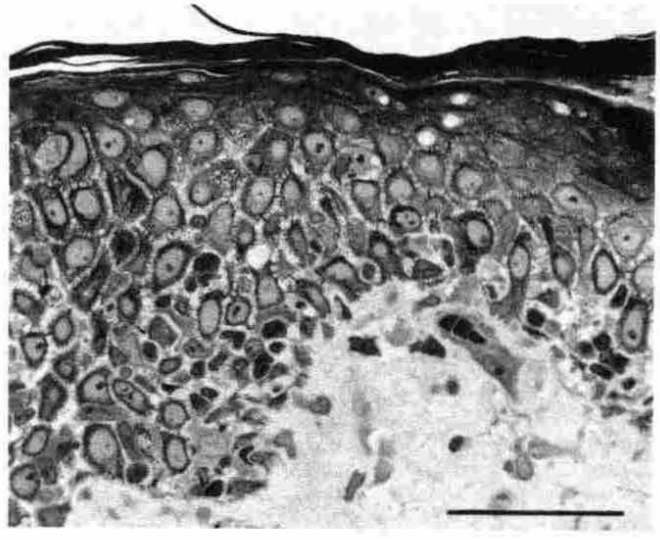

Figure 6. Mild spongiosis with exocytosis of mononuclear cells in benzalkonium chloride reaction. Toluidine blue-stained 1-μm semithin section (*bar* = 50 μm).

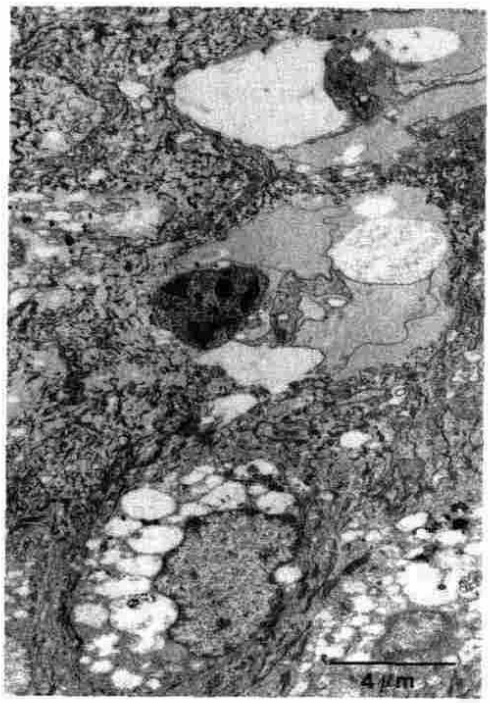

Figure 7. Ultrastructural appearance of necrotic damage caused by benzalkonium chloride. The cells have pyknotic nuclei and disrupted membranes and organelles, with considerable intracytoplasmic vacuolation.

nuclei. In a few cells the nuclei were shrunken and pyknotic and accompanied by marked perinuclear vacuolization. Keratinocytes in the stratum spinosum and basal epidermis frequently contained large numbers of lipid droplets.

DISCUSSION

We have studied the histopathology of irritant contact dermatitis reactions elicited in human skin by a range of chemically unrelated irritants. Several of these induced patterns of cellular damage in the epidermis, which were consistent among individuals despite variations in their intensity of response, and, in some cases, characteristic of that particular chemical. A notable example of this was the 9-carbon chain fatty acid nonanoic acid [21], which caused distinct histopathological changes to keratinocytes in the upper epidermis, closely resembling those described in early lesions of necrolytic migratory erythema (E. Wilson Jones, personal communication). On ultrastructural examination dense aggregates of keratin filaments were found to be present in the cytoplasm, suggestive of premature keratinization. Disturbances in epidermal cell differentiation may therefore have been induced, although irreversible cellular injury may give rise to a similar appearance [22]. Unlike the findings of Dowd et al [23], on patch testing with 40% nonanoic acid for 24 h, only minimal spongiosis was produced, even in those reactions judged to be moderate on the basis of erythema, edema etc.

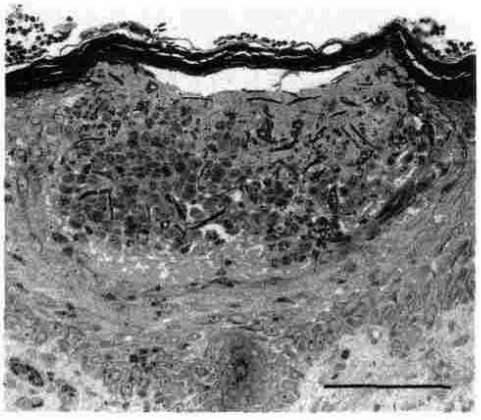

Figure 8. Intraepidermal bulla containing polymorphonuclear leukocytes, mononuclear cells, and acantholytic keratinocytes produced in a severe reaction to croton oil. Toluidine blue-stained 1-μm semithin section (*bar* = 100 μm).

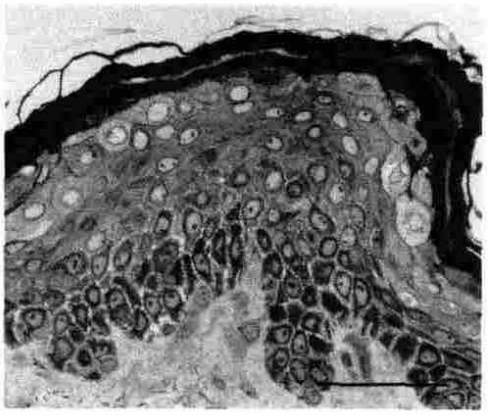

Figure 9. Moderate dithranol reaction showing marked swelling of keratinocytes in stratum granulosum and upper stratum spinosum. Toluidine blue-stained 1-μm semithin section (*bar* = 50 μm).

The anionic detergent, sodium lauryl sulphate, in common with nonanoic acid, also caused profound alterations to the normal morphology of keratinocytes, including marked parakeratosis in the majority of biopsies. This may have been due to increased epidermal cell turnover, as sodium lauryl sulphate has been shown to stimulate epidermal mitosis [24]. Alternatively, it may signify accelerated keratinization or direct cytotoxic injury [22]. In addition to parakeratosis, sodium lauryl sulphate induced considerable vesiculation and lipid accumulation within the cytoplasm of keratinocytes—morphologic features that are indicative of disturbed metabolic activity, and that were similarly described by Tovell et al [25] after repeated application of 1% sodium lauryl sulphate to rat skin.

Patch testing with the cationic detergent, benzalkonium chloride, produced inflammatory changes that differed from those of

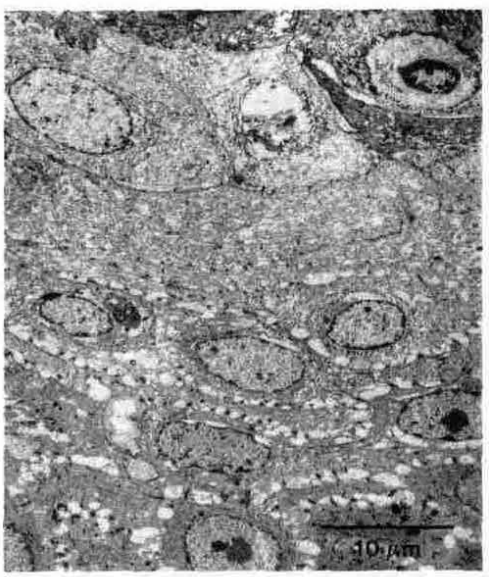

Figure 10. Transmission electron micrograph of enlarged upper epidermal keratinocytes in dithranol reaction. The cytoplasm contains finely dispersed filaments and ribosomes with few organelles or keratohyalin granules.

sodium lauryl sulphate, a contrary finding to that of Gisslén and Magnusson [26]. Parakeratosis and the accumulation of lipid were not seen but, unlike sodium lauryl sulphate reactions, necrotic damage was evident, even in those patch tests judged clinically to be mild, suggesting different mechanisms of skin irritation between the two types of detergent.

Cellular damage to keratinocytes was also evident in patch test reactions to the antipsoriatic drug, dithranol. Mild responses showed a nonspecific spongiosis and exocytosis but, in the majority of those assessed as moderate, marked swelling or ballooning of keratinocytes occurred in the upper epidermis, similar to that associated with cutaneous viral infections [27]. Such an effect may be associated with one of dithranol's known cytotoxic properties, that of interference with mitochondrial function [28,29].

Application of the hygroscopic agent, propylene glycol, failed to produce significant irritation to the skin, in contrast to the findings of Nater et al [30]. However, a distinctive basketweave pattern to the stratum corneum was seen in all samples, due to the osmotic hydration of corneal cells [31].

Croton oil elicited inflammatory responses which perhaps most closely resembled those of allergic contact dermatitis [20,32]. Marked spongiosis, accompanied by considerable exocytosis, was a feature of almost all patch test reactions, including those assessed visually to be mild. Morphologic evidence of disturbances in keratinocyte metabolism or kinetics was rarely observed, suggesting a primary effect on leukocytes, possibly chemotactic in nature.

We have concentrated primarily on the cellular changes that occurred in the epidermis after topical application of irritants. Characterization of the inflammatory cell populations that infiltrated the skin and participated in these reactions is currently being undertaken. It is interesting to note, however, that mononuclear cells were the predominant responding cells in all reactions judged to be mild or moderate. Significant numbers of polymorphonuclear leukocytes were present only in the three severe reactions to croton oil and sodium lauryl sulphate, suggesting that the inflammatory mediators released when severe tissue damage occurs differ from those produced when the pathological changes are of a milder nature.

It is highly likely that the patterns of cellular damage that we observed in the epidermis are dose as well as time dependent. If applied at concentrations above certain threshold levels, many irritants will undoubtedly cause overwhelming tissue damage, obscuring the more subtle morphologic indications of disturbances in epidermal cell metabolism. For mild and moderate reactions, however, our results demonstrate that the histopathological changes occurring in irritant contact dermatitis vary in accordance with the nature of the irritant, reflecting their different chemical interactions with the skin.

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